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Influence of selected wound dressings on PMN elastase in chronic wound fluid and their antioxidative potential in vitro

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Abstract

Exudates from non-healing wounds contain elevated levels of proteolytic enzymes, like elastase from polymorphonuclear granulocytes (PMN elastase), reactive oxygen species (ROS) and reactive nitrogen species (RNS). The overproduction of proteolytic enzymes leads to reduced concentrations of growth factors and proteinase inhibitors, resulting in an imbalance between degradation and remodelling processes. Thus, the reduction of protein-degrading enzymes and scavenging of ROS and RNS seem to be suitable ways to support the healing process of chronic stagnating wounds. The aim of this study was to test selected wound dressings from different biomaterials (collagen, oxidized regenerated cellulose (ORC) and ORC/collagen mixture), regarding their antioxidative potential in vitro and their influence on the concentration and activity of PMN elastase in chronic wound fluid. Antioxidant capacity of the investigated wound dressing was determined by a pholasin-based chemiluminescent assay. PMN elastase concentration was determined by means of ELISA. Enzyme activities could be measured by a fluorescence assay. As the presented data demonstrates, all tested materials showed antioxidant capacity. In addition, the investigated materials were able to reduce the concentration and activity of PMN elastase. Beside other aspects, such as biocompatibility, biodegradability, fluid absorption and clinical effects (e.g. angiogenesis and microcirculation), the understanding of these properties may help to support the further refinement of wound dressings for improved wound healing.

Keywords: Collagen; Cellulose; Wound dressing; PMN elastase; Free radicals; Wound healing

1. Introduction

Chronic wounds are an important and persistent problem in dermatology. Non-healing chronic wounds, e.g. diabetic or venous ulcers, differ from acute wounds significantly. Whereas healing acute wounds have low levels of protein-degrading enzymes, exudates from non-healing chronic wounds contain elevated levels of proteases, like matrix metalloproteinases (MMPs) and elastase [1–3]. Moreover, the concentrations of pro-inflammatory cytokines [4], as well as reactive oxygen species (ROS) [5] are significantly higher, compared to the concentrations in acute wounds. Various triggers contribute to this particular microenvironment of chronic wounds, e.g. ischemia, neuropathy and infection.

Normal wound healing is a dynamic process following three phases, inflammation, granulation (tissue formation) and re-epithelization (tissue remodelling), which

Abbreviations: PMN granulocytes, polymorphonuclear granulocytes; ROS, reactive oxygen species; RNS, reactive nitrogen species; ORC, oxidized regenerated cellulose

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are overlapping in time [6]. The healing mechanism involves various cell types, biochemical factors and extracellular matrix molecules [7]. Subsequent to an injury, a fibrin clot serves as a temporary shield protecting the wounded tissue. Blood clotting also induces the degranulation of platelets that release various cytokines and growth factors into the surrounding tissue, i.e. platelet-derived growth factor (PDGF), transforming growth factor β (TGF-β) and epidermal growth factor (EGF) [8]. Inflammatory cells, such as polymorphonuclear (PMN) granulocytes, migrate into the wound followed by macrophages. PMN granulocytes release proteases including PMN elastase [3] and collagenase (MMP-8) [3,9]. Furthermore, ROS, such as superoxide radicals ($O_2^{\bullet -}$) and hydroxyl radicals ($^{\bullet}OH$), as well as reactive nitrogen species (RNS), such as nitric oxide ($NO^{\bullet}$), arise from inflammatory cells [10–12]. Activated macrophages destroy bacteria and liberate inflammatory cytokines, as tumor necrosis factor α (TNF-α) and interleukin 1β. Under physiological conditions, the number of PMN granulocytes decreases after a few days. However, in chronic wounds, several authors have shown a massive and constant infiltration of PMN granulocytes [2,13]. The excessive production of elastase and other proteolytic enzymes by PMN granulocytes leads to considerably reduced amounts of growth factors [14] and proteinase inhibitors [15]. PMN elastase has been identified as one of the major enzymes responsible for degradation of peptide growth factors [15]. In addition, overproduction of ROS and RNS results in an imbalanced oxidant/antioxidant status in chronic wounds [5,12,16]. Due to the resulting disproportion between degradation and remodelling processes, chronic wounds persist in the inflammatory phase of the normal healing process and often remain non-healing for months or even years. Therefore, the reduction of ROS/RNS and PMN elastase in the wound fluid seems to be a suitable way to stop the vicious circle of inflammation and diminished epithelization and thus to support the normal wound healing process.

The aim of the presented study was to test selected wound dressings of different compositions, regarding their influence on PMN granulocyte products. In particular, the effects of these wound dressing materials on ROS/RNS and PMN elastase, as specific parameters of PMN granulocytes, were studied.

Current concepts of modern wound management are focused on a moist wound environment. Since the middle of the 20th century, several studies have shown that occlusive dressings are beneficial to re-epithelization of wounds [17,18]. Today, a variety of occlusive dressings are on the market in different forms, including films, foams and gels, and from diverse materials as alginates, polyurethane or hyaluronic acid [17]. In the presented study, wound dressings from different biomaterials were included: a collagen foam product consisting of collagen type I, from bovine origin (Suprasorb® C), a dressing from oxidized regenerated cellulose (ORC, Tabotamp®), as well as a mixture from collagen and ORC (Promogran®). Suprasorb® C is a flexible, porous foam product with high capillary activity. Collagen can absorb large quantities of fluids and it forms a soft gel that keeps its environment moist [19]. Furthermore, collagen provides haemostatic properties [20,21]. Thus, Suprasorb® C is eligible for applications in the maintenance of non-healing chronic wounds. Promogran®, a dressing consisting of a sterile, freeze-dried matrix, is composed of collagen and ORC, and combines the properties of its components, such as fluid absorption and haemostatic properties. Uses of Promogran® in the treatment of diabetic foot ulcers and venous leg ulcers have been described [22–25]. Tabotamp® is a thin gauze layer from pure ORC. ORC is in use in acute wounds like trauma [26], surgical injuries [27] and burns [28]. It provides haemostatic [21] and antimicrobial properties [29].

Bacterial cellulose, which has been proven to be a rather inert material, was chosen as a negative control. It finds a wide range of applications in medicine, e.g. as a temporary skin substitute [30–32] or as a wound dressing for burns [33].

2. Materials and methods

2.1. Materials

Suprasorb® C was obtained from Lohmann & Rauscher GmbH & Co. KG (Rengsdorf, Germany). Promogran® was purchased from Johnson & Johnson MEDICAL Limited (Gargrave, UK). Tabotamp® was provided by ETHICON SARL (Neuchâtel, Switzerland). Bacterial cellulose was synthesized using the *Acetobacter xylinum* strain AX5 (DSMZ laboratory subspecies), cultivated in liquid Hestrin–Schramm medium [34] for 14 days. The cellulose pellicle that formed during that time at the air/liquid interface was harvested, sterilized at 121 °C for 20 min, treated with 0.1 N NaOH for 30 min at 100 °C and, afterwards, washed with distilled water to neutral pH. The pellicles were freeze dried for 48 h before usage.

Human PMN elastase standard (1 μg/mL) was taken from an Enzyme Immunoassay (ELISA), purchased from Milenia Biotech (Bad Nauheim, Germany). Elastase from pig pancreas was used from the EnzChek Elastase Assay Kit (MoBiTec, Goettingen, Germany). Chronic wound fluid was obtained from a 50-year-old male patient suffering from venous insufficiency and peripheral occlusive disease. He was treated with a V.A.C.® vacuum pump (Kinetic Concepts Inc., San Antonio, USA) to remove fluid from the wound. The total protein content of the collected wound fluid was 6.4 ± 1.1 mg/mL. Wound fluid was aliquoted and stored at −80 °C, until required for further experiments.

2.2. Sample preparation

The lyophilized PMN elastase standard (Milenia, Bad Nauheim, Germany) was reconstituted with the calibrator sample diluent, as recommended. For further experiments the reconstituted elastase standard was diluted to a final concentration of 250 ng/mL. A 100 U/mL stock solution of elastase from pig pancreas (MoBiTec, Goettingen, Germany) was prepared in distilled water. Aliquots were frozen and stored at −20 °C. For experiments, the stock solution was diluted to 0.1 U/mL in the reaction buffer (0.1 M Tris–HCl, pH 8.0, containing 0.2 mM sodium azide and 0.5% bovine serum albumin).

Wound fluid was diluted 100-fold in phosphate-buffered saline prior to incubation. Samples of collagen, ORC/collagen, ORC and bacterial cellulose were cut using 8 mm biopsy punches (Stiefel Laboratorium GmbH, Offenbach, Germany) corresponding to 0.5 cm^2. For antioxidant capacity measurements, the samples were further sliced into half and quarter pieces. The samples were pre-wetted with phosphate-buffered saline (pH 7.4) and placed into 24-well cell culture plates. Each specimen was included in a final volume of 1 mL of enzyme solution or wound fluid. Samples were incubated up to 24 h at 37 °C on a plate mixer (THERMOstar, BMG Labtech GmbH, Offenburg, Germany). After incubation, samples were collected, immediately frozen and stored at −20 °C until required.

2.3. Determination of antioxidant capacity

The capability of the tested wound dressings to scavenge ROS and RNS was assessed using chemiluminescent ABEL® Antioxidant Test Kits specific for peroxynitrite and ROS, such as superoxide radical species. Both test kits contain Pholasin®. Both test kits were purchased from Knight Scientific Limited (Plymouth, UK). Pholasin® is a photoprotein, isolated from the mollusc *Pholas dactylus*, which emits light in the presence of certain oxidants. As the tests are based on cell independent systems, with the kits the necessary solutions to create reactive molecules that activate the Pholasin® are provided.

Peroxynitrite is formed by the reaction of superoxide and nitric oxide, released from a 2.5 mM solution of SIN-1 (3-morpholino-sydnomine HCl). Measurements were run in 96-well microplates (white, flat bottomed, Nunc, Denmark) and carried out as recommended in the kit instructions in a total reaction volume of 200 μL. Samples were placed into the microplate. To each sample, 100 μL assay buffer and 50 μL Pholasin® were added. At the start of the measurement, 50 μL SIN-1 solution was injected to each well with an automatic dispenser. The luminescence was measured for up to 2 h, depending on the peak time of the different samples, at room temperature using the LUMIstar Galaxy plate reader (BMG Labtech GmbH, Offenburg, Germany). A control without sample was run with each assay. An antioxidant activity delays the appearance of the luminescence peak and lowers its light intensity. The antioxidant capacity of a sample was expressed as per cent reduction of peak luminescence as follows:

$$\frac{[(\text{peak, control}) - (\text{peak, sample})]}{(\text{peak, control})} \times 100.$$

The chemiluminescent test for superoxide was carried out according to the kit protocol in 96-well microplates (white, flat bottomed, Nunc, Denmark) at room temperature. The samples were added to 25 μL assay buffer, 50 μL Pholasin® and 100 μL Solution A. While the well was in the light measuring position, 25 μL Solution B was injected to generate a superoxide flux. As the peak of the light intensity was reached within 5 s, the LUMIstar Galaxy plate reader (BMG Labtech GmbH, Offenburg, Germany) was programmed to measure each well for 30 s, in short intervals after the injection of Solution B.

The antioxidant capacities of the samples were calculated similar to the peroxynitrite test.

2.4. Determination of PMN elastase

An enzyme immunoassay for the quantitative measurement of human elastase from PMN granulocytes was purchased from Milenia Biotech (Bad Nauheim, Germany). The assay was run as recommended in the instructions. Briefly, 100 μL of calibrators, controls and samples was added to the microplate and incubated for 1 h at room temperature. After sample aspiration, the plate was washed four times and 150 μL antibody conjugate solution, labelled with horseradish peroxidase, was added to each well. Again, the microplate was incubated at room temperature for 1 h. Subsequently, the plate was washed four times and 200 μL 3,3′,5,5′-tetra-methylbenzidine substrate solution was added. After incubation in the dark for 20 min, the reaction was stopped by adding 50 μL of 2 M HCl solution. Optical density was measured at 450 nm with a reference measurement at 620 nm. Subsequently, the PMN elastase concentration was evaluated according to a 4-Parameter-Fit with lin–log coordinates for optical density (linear scale) and concentration (logarithmic scale).

2.5. Determination of enzymatic elastase activity

The EnzChek Elastase Assay Kit was purchased from MoBiTec (Goettingen, Germany). The EnzChek Kit contains a fluorescence-labelled elastin substrate (*DQ elastin* from bovine neck filament; 4,4-difluoro-5,7-dimethyl-4-bora-3a,4a-diaza-*s*-indacene-3-propionic acid), which can be digested by elastase to yield fluorescent products. Fluorescence was determined at 538 nm. The fluorophore was excited at 495 nm. Elastase from pig pancreas was used as calibrator substance. This enzyme differs slightly from human PMN elastase regarding its specificity for synthetic substrates. Since the fluorescent DQ elastin substrate, used in the EnzChek Elastase Assay Kit, is optimized for pancreatic elastase, this enzyme was selected as enzyme standard. Measurements were run in 96-well microplates (black, flat bottomed, Nunc, Denmark). To 50 μL reaction buffer, 50 μL DQ elastin substrate (100 μg/mL) was added followed by the injection of 100 μL sample solution. The fluorescence was measured continuously for 1 h, at room temperature, using the POLARstar plate reader (BMG Labtech GmbH, Offenburg, Germany).

2.6. Statistical analysis

Studies were performed in duplicate, and each sample was measured in two replicas. All values cited are means from at

least four data points. One-way analysis of variance (ANOVA) was carried out to identify statistical significances (Microsoft® Excel 2000).

3. Results

3.1. Antioxidant capacity measurement

All tested wound dressings showed antioxidant capacity, but ORC was the most effective. Indeed, even 0.12 cm^2 of the dressing showed a significant reduction ($p<0.001$) of almost 100% of the peak intensity in the tests for peroxynitrite and superoxide radical species (data not shown). The ORC/collagen dressing showed comparable results of nearly complete superoxide scavenge at 0.5 and 0.25 cm^2 samples ($p<0.001$), as illustrated in Fig. 1. ORC/collagen was also very effective against peroxynitrite ($p<0.001$), performing 74% (0.25 cm^2) and 91% (0.5 cm^2) radical scavenging efficiency (Fig. 2). Collagen type I and bacterial cellulose were less effective compared to ORC and ORC/collagen. Nevertheless, both dressings were able to scavenge both peroxynitrite and superoxide radical species (Figs. 1 and 2).

3.2. Concentration of unbound PMN elastase in a defined enzyme solution

As shown in Fig. 3a, collagen type I reduced the concentration of residual PMN elastase in a time-dependent manner. Incubation with 0.5 cm^2 pieces of the collagen product resulted in a significant reduction of unbound PMN elastase after 6 h ($p<0.05$). Same sized pieces of the ORC/collagen dressing did not affect the concentration of the free enzyme (Fig. 3b, white circles). A similar effect could only be found for the ORC/collagen product at higher amounts of the applied material. The wound dressing from pure ORC caused the most pronounced effect. It significantly reduced the concentration of unbound elastase after 30 min ($p<0.001$), as shown in Fig. 3c. Bacterial cellulose did not influence the concentration of unbound PMN elastase (Fig. 3d).

3.3. Concentration of unbound PMN elastase in a wound fluid sample

Comparable results as for the enzyme solution were found in the wound fluid sample. Collagen type I did bind significant quantities of PMN elastase from the residual solution (Fig. 4a). After 6 h, the amount of unbound elastase was reduced to about 35% ($p<0.01$). As in the enzyme solution, the ORC/collagen dressing was only effective at higher amounts of the applied material (Fig. 4b). The ORC product did bind PMN elastase most efficiently (Fig. 4c). After 30 min, the protease was completely removed ($p<0.005$). Again, bacterial cellulose had no effect (Fig. 4d).

3.4. Enzymatic activity of elastase

The binding of elastase from an enzyme solution or wound fluid is accompanied by a significant loss in

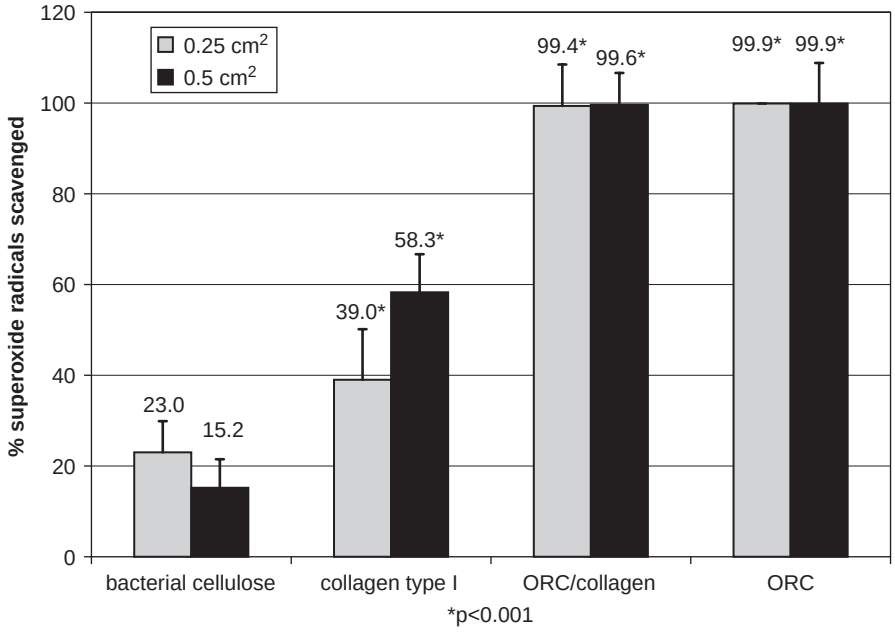

Fig. 1. Dose-dependent scavenging of superoxide radicals ($O_2^{\bullet -}$) by bacterial cellulose, collagen I, ORC/collagen and ORC. Data are specified as mean ± SE.

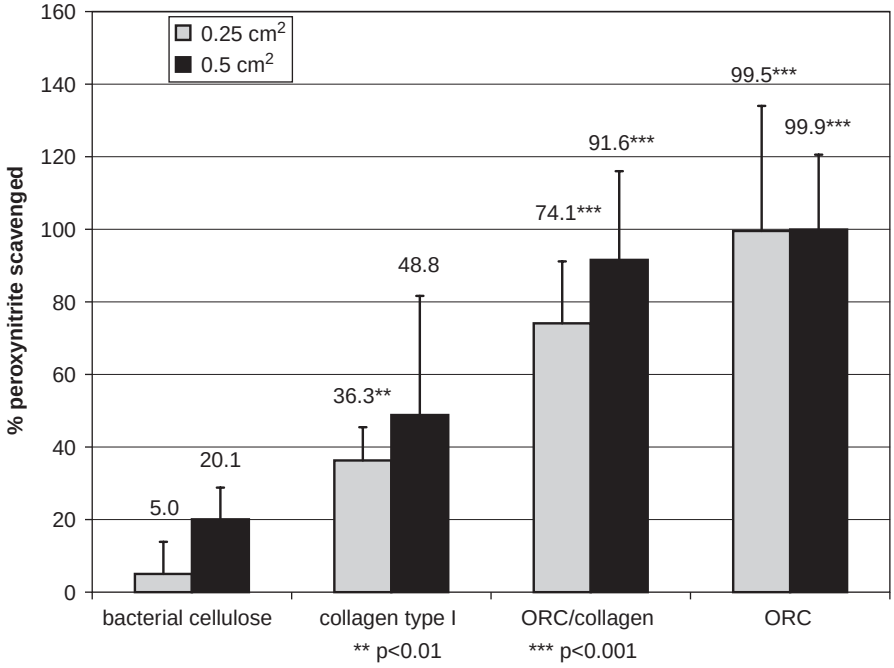

Fig. 2. Dose-dependent scavenging of peroxynitrite ($ONOO^-$) by bacterial cellulose, collagen I, ORC/collagen and ORC. Data are specified as mean ± SE.

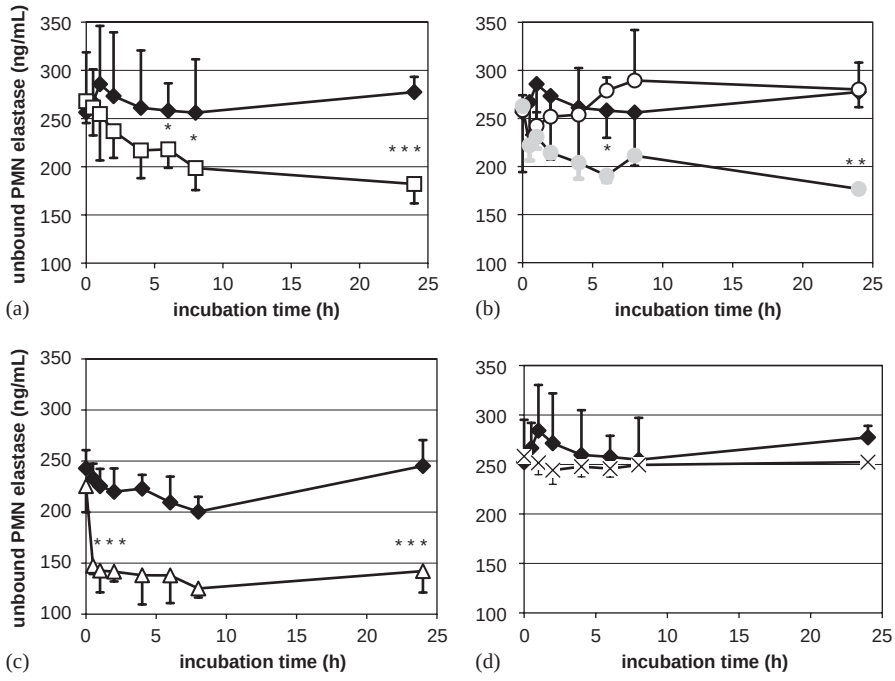

Fig. 3. Binding of PMN elastase by collagen I (a), ORC/collagen (b), ORC (c) and bacterial cellulose (d) from an enzyme solution. ◆, control; □, 0.5 cm^2 collagen type I; ○, 0.5 cm^2 ORC/collagen; ●, 1.0 cm^2 ORC/collagen; △, 0.5 cm^2 ORC; ×, 0.5 cm^2 bacterial cellulose. Data are specified as mean ± SD. $^*p<0.05$, $^{**}p<0.01$ and $^{***}p<0.001$.

enzymatic activity. As Fig. 5 shows, the ability of elastase to digest the fluorescence substrate decreased with increasing incubation time. Collagen type I and the ORC/collagen showed similar capabilities to inhibit elastase activity as illustrated in Fig. 5a and b. Both dressings reduced the enzymatic activity of elastase after 6 h of incubation significantly ($p<0.005$ and <0.001, respectively). The product from pure

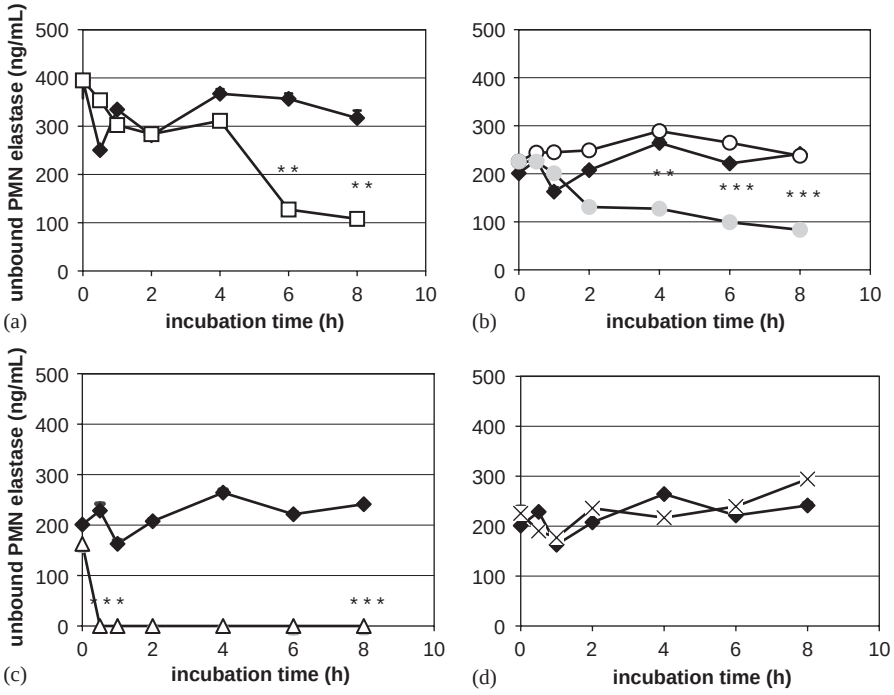

Fig. 4. Binding of PMN elastase by collagen I (a), ORC/collagen (b), ORC (c) and bacterial cellulose (d) from chronic wound fluid. ◆, control; □, 0.5 cm^2 collagen type I; ○, 0.5 cm ^{2} ORC/collagen; ●, 1.0 cm^2 ORC/collagen; △, 0.5 cm^2 ORC; ×, 0.5 cm^2 bacterial cellulose. Data are specified as mean ± SD. $^*p<0.05$, $^{**}p<0.01$ and $^{***}p<0.001$.

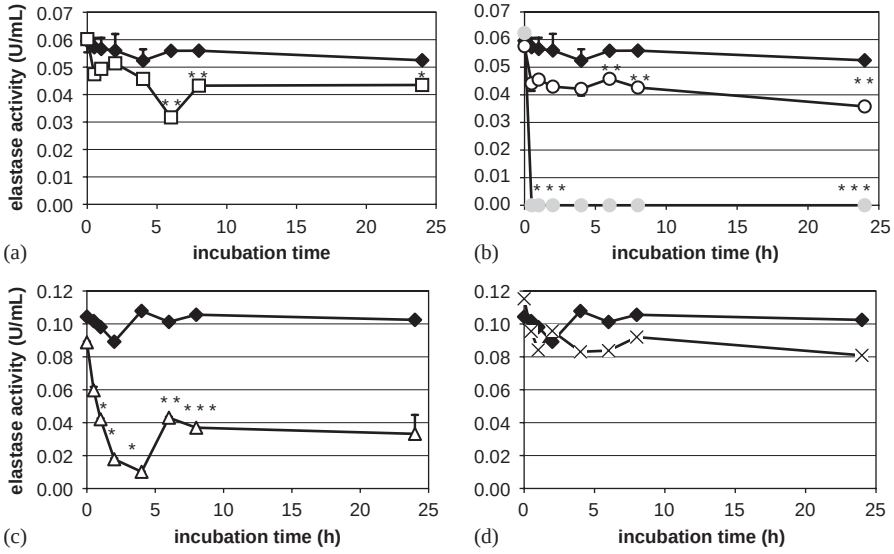

Fig. 5. Reduction of elastase activity by collagen I (a), ORC/collagen (b), ORC (c) and bacterial cellulose (d). ◆, control; □, 0.5 cm^2 collagen type I; ○, 0.5 cm ^{2} ORC/collagen; ●, 1.0 cm^2 ORC/collagen; △, 0.5 cm^2 ORC; ×, 0.5 cm^2 bacterial cellulose. Data are specified as mean ± SD. $^*p<0.05$, $^{**}p<0.01$ and $^{***}p<0.001$.

ORC was most effective (Fig. 5c). A significant inhibition was achieved already after 30 min ($p<0.005$). Bacterial cellulose did not influence the elastase activity (Fig. 5d).

4. Discussion

ROS and RNS, such as superoxide radicals ($O_2^{\bullet -}$), hydroxyl radicals ($^{\bullet}OH$) and nitric oxide ($NO^{\bullet}$),

arise from inflammatory cells, which are strongly implicated in the pathogenesis of several diseases including chronic ulcers [35–37]. While ROS/RNS play an important role in the normal wound healing process by killing invading microorganisms, the excessive overproduction of these species causes indiscriminate cellular damage. As studies with human dermal fibroblasts have shown, ROS induce increased concentration of MMPs on mRNA level [38]. Consequently, the scavenging of ROS has been proven to be beneficial for wound healing [39].

As the presented data show, the ability of the tested wound dressings to scavenge ROS and the RNS, peroxynitrite ($ONOO^-$), varies to a great extent. The ORC product and the mixture from ORC/collagen showed a nearly complete depletion of ROS and RNS. In contrast, pure collagen I resulted in a significantly lesser ability to deplete both ROS and RNS, compared to ORC and ORC/collagen. However, the effect of collagen I was clearly more pronounced than the effect of the bacterial cellulose, which served as a negative control. With respect to the different scavenge effects of collagen and ORC/collagen, quite opposing results were obtained in another study, where a comparable scavenging ability of collagen type I (Suprasorb® C) and ORC/collagen (Promogran®) for ROS has been shown [40].

However, since the analytical methods for the ROS differed between the two studies, further investigations are needed to describe the effects in more detail.

Nevertheless, the proven high ability of ORC to act against superoxide radical species and peroxynitrite seems likely to be due to the numerous functional groups, such as OH and COOH, offering a variety of sites for radical attack leading to electron and hydrogen abstraction from α-carbons. Furthermore, as various sugars and other polyols, ORC could be able to suppress the metal-catalysed oxidation of proteins by complexation of metal ions [41]. In addition, studies have demonstrated the ROS scavenging capacity of comparable wound materials such as carboxymethylcellulose, hyaluronan and hyaluronan benzyl esters [42,43]. The observed antioxidant capacity of collagen type I can be attributed to the amino acid residues like lysine and proline, which have been reported to be highly susceptible to ROS attack [44–46]. In addition, ROS-induced modification and loss of functional groups of other amino acids, such as methionine, histidine and tyrosine, have been reported [47]. Due to the interaction of these amino acids within the polypeptide chains with ROS, collagen exhibits radical scavenger properties as well. In addition, a binding capacity for metal ions has been observed for collagen type I [40].

Impaired wound healing results from an imbalance between degradation and remodelling processes. While proteolytic enzymes, such as PMN elastase and MMPs, are overexpressed in chronic wounds, the concentration and activity of essential growth factors (e.g. PDGF and KGF) are significantly decreased. Numerous clinical studies using recombinant growth factors to improve the healing of chronic wounds have been performed in recent years [48,49]. Topical application of growth factors has proven to be effective in chronic wound healing, but requires large and repeated doses of the applied factor.

As long as the protease concentration is abounding in the chronic wound, the response to growth factor therapy remains limited [50]. Studies were undertaken in order to inhibit elastase activity by a wound dressing formulated with an elastase inhibitor [51] and to bind elastase specifically by immobilization of elastase recognition sequences on cotton gauze [52]. As the results of this study demonstrate, different biomaterials are able to bind PMN elastase from enzyme solutions and in chronic wound fluid in a time-dependent manner. However, the in vitro binding capacities of the investigated dressings varied considerably. In the solution of PMN elastase, as well as in samples from wound fluids, the pure ORC showed the greatest effect, with a significant reduction of the unbound PMN elastase in the enzyme solution and a complete elimination of the elastase in the wound fluid. A comparison between the collagen product and the ORC/collagen combination demonstrated a higher binding capacity for collagen, compared to ORC/collagen. Whereas collagen reduced the PMN elastase concentration significantly in both solutions after 6 h of incubation, the comparable sized samples of ORC/collagen did not show any significant reduction at all. However, a comparable reduction of unbound elastase was observed, when the size of the tested collagen/ORC pieces was increased to 1 cm^2.

In order to explain the varying binding capacities, a closer look at the structure of the biomaterials is needed. ORC contains a large number of functional groups, such as OH, CHO, CO, COOH, which allow various interactions with other molecules via hydrogen bonds and electrostatic interactions. As studies with oxidized, phosphorylated, carboxymethylated and sulphonated cotton-gauze have shown, the chemical modification of the wound dressing does lead to a decreased elastase activity in chronic wound fluids [53]. The authors suggested that the negatively charged groups of the gauze interact with arginine side chains on the surface of elastase.

Pure collagen type I also reveals efficient elastase binding capability. Collagen dressings are widely used as beneficial covers for chronic wounds, because of their ability to keep the wound climate moist. Its porous structure and capillary activity allow collagen to absorb large quantities of fluid [19]. Furthermore, collagen

provides physical support for cellular proliferation [54], is biocompatible and biodegradable. The fibrillar collagen molecules within the foam product build a super-coiled triple helical structure, which is based upon non-covalent interactions between vicinal polypeptide strains [55]. According to the typical amino acid composition of collagen type I, which consists of glycine as every third residue and large amounts of proline, hydroxyproline and hydroxylysine, the polypeptide chains build intra- and intermolecular cross-links. Several authors have shown that collagen fibres are stabilized as well by formation of electrostatic interactions and hydrophobic stabilization [56,57]. However, few of the backbone groups are available for additional interaction with other molecules. For example, the electrostatic interactions between collagen and chitosan [58], poly(acrylic acid) [59] and hydrophilic monomer ligands [60] have been demonstrated. Thus, it seems likely that the collagen wound dressing binds PMN elastase by non-covalent interactions. The high capillary activity of the collagen foam could support the absorption of proteins into the three-dimensional structure of the dressing.

Interestingly, the wound dressing mixed from ORC and collagen exhibited remarkable less elastase binding capacity than its single components. It seems that due to the combination of both ORC and collagen, the potential for electrostatic interactions with other molecules is partly impaired. However, the reduction of elastase activity by collagen/ORC was comparable with the effect of equal sized pieces of pure collagen type I. The ORC/collagen dressing was subject for several studies regarding its efficiency to promote wound healing [25,61]. ORC/collagen reduces collagenase as well as MMP-2 and MMP-9 activities in the wound fluid obtained from diabetic foot ulcers [62] and was found to promote human dermal fibroblasts proliferation [63].

The definite mechanism of elastase inactivation by the investigated wound dressings has not been clarified so far. It seems likely that sterical hindrance of the active site of the enzyme due to the binding is one possible mechanism of elastase inactivation. Additionally, reduced substrate accessibility as a consequence of the interaction with the wound dressing could also contribute to the effect.

The results in this study impressively demonstrate the value of in vitro experiments with respect to the influence of wound dressings on single factors in the wound healing process. Nevertheless, it has to be mentioned that wound healing is rather complex. Beside a binding capacity for wound fluid components that hinder the healing process, positive effects of wound dressings, such as the stimulation of angiogenesis and microcirculation [64,65] of the wound bed, also have to be taken into consideration.

5. Conclusions

The data presented herein provide rational information on the effects of topical dressings on specific parameters in chronic wounds. Due to their composition, the investigated wound dressings showed different abilities to bind and inactivate PMN elastase, as well as ROS and RNS. Beside other aspects, such as biocompatibility, biodegradability, fluid absorption and clinical effects (e.g. angiogenesis and microcirculation), the understanding of these properties may help to support the further refinement of wound dressings for improved wound healing.

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